Ultrasound Strain Rate Imaging in Functional Dyspepsia

In-vitro and clinical evaluation

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To the soul of my father in its heavenly existence

To my mother: for her great sacrifices and love...

To my beloved wife Muna for her love and patience...

To our children: Ahmed, Ashraf and Sara:

For all the joy they brought to our life...

And for lovely expectations...

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Abstract

Functional dyspepsia (FD) is a chronic condition that is characterized by abdominal pain or discomfort, nausea, vomiting, early satiation and abdominal fullness. The Rome III classification of functional gastrointestinal disorders defines two distinct entities of functional dyspepsia (FD), namely “epigastric pain syndrome” (EPS) and “postprandial distress syndrome” (PDS). Strain rate imaging (SRI) is a new ultrasound modality that is used to give a quantitative measure of deformation. The method was developed to study the cardiac muscle during health and disease.

In this doctoral thesis we used SRI to study antral contractions in healthy volunteers and in patients with FD. The original cardiac SRI method was first adjusted to be able to detect signals from slowly contracting tissues. The accuracy of the adjusted method was tested in a rubber phantom mimicking the antral muscular wall in thickness, homogeneity and contraction velocity (paper I). We found that SRI strains agreed well with calculated values for strain when SRI strain was measured as the average over several US beams and the strain sample size was 1.9 mm. The variance was substantial if single samples were used, especially for a small strain sample size (0.8 mm). The results showed low inter and intraobserver variation. With the new parameters acquired from that study, the method was then applied in an ex-vivo porcine antral wall (paper II). The agreement between radial strain values measured by SRI and B-mode, along and across several ultrasound beams, using US frequency 6.7 MHz and strain length (SL) = 1.9 mm was good and it was better than with SL 1.2 mm. The method was then applied on the stomachs of healthy individuals (paper III).

Erythromycin, a prokinetic agent, was also used to induce contractions. SRI enabled detailed strain estimation of individual antral contractions and the typical motility pattern during fasting that includes a migrating motor complex activity was recognised. Erythromycin influenced antral contractility and increased the number of lumen occlusive contractions.

The method was then applied in a cohort of patients with FD and healthy volunteers. FD patients were allocated one of FD subgroups: EPS or PDS. Antral strain in EPS patients was significantly higher than both in controls and PDS patients during fasting and postprandially. PDS patients had lower strain than controls during fasting, but not postprandially. The area of the proximal stomach during fasting was significantly larger in FD than in controls. The accommodation response ratio was significantly higher in controls than in FD. Gastric emptying fraction at 20 and 40 min postprandially was significantly larger in controls than in FD. The symptom score in the fasting state and the area under the curve in the postprandial state were significantly higher in FD patients than in HC for all the symptoms.

We conclude that SRI can be a valuable method to noninvasively quantify antral contractions and that anterior radial strain measured by SRI can discriminate between HC and subgroups of FD.

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2.0 Acknowledgement

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Last, but not least, I would like to express my deepest gratitude to my beloved companion and friend, my wife Muna for her support, tolerance and devotion.

3.0 Abbreviations

CSA: cross-sectional area

EPS: epigastric pain syndrome

FD: functional dyspepsia

MMC: Migrating Motor Complex

MRI: Magnetic Resonance Imaging

PDS: postprandial distress syndrome

SRI: Strain Rate Imaging

TVI: Tissue Velocity Imaging

4.0 List of papers

This dissertation is based on the following papers, referred to in the text by their roman numerals

Paper I

Matre K, Ahmed AB, Gregersen H, Heimdal A, Hausken T, Ødegaard S and Gilja OH. **In vitro evaluation of ultrasound Doppler strain rate imaging: modification for measurement in a slowly moving tissue phantom.** *Ultrasound Med Biol* 29: 1725-34, 2003.

Paper II

Ahmed AB, Gilja OH, Gregersen H, Ødegaard S and Matre K. **In vitro strain measurement in the porcine antrum using ultrasound Doppler strain rate imaging.** *Ultrasound Med Biol* 32: 513-22, 2006.

Paper III

Ahmed AB, Gilja OH, Hausken T, Gregersen H and Matre K. **Strain measurement during antral contractions by ultrasound strain rate imaging - Influence of Erythromycin.** *Neurogastroenterol Motil, in press- available online.*

Paper IV

Ahmed AB, Matre K, Hausken T, Gregersen H and Gilja OH. **Rome III Subgroups of functional dyspepsia exhibit different characteristics of antral strain measured by strain rate imaging**. *Neurogastroenterol Motil, submitted.*

5.0 Introduction

5.1. Medical ultrasonography:

Medical ultrasound (US) is a modality used for non-invasive visualisation of internal organs. US has become a wide-spread clinical tool that helps in the diagnosis and management of a variety of medical conditions. It is applied in virtually all clinical specialities.

The physics of US

While the human ears can perceive sound waves in the range of 20-20000 Hz, medical US uses frequencies of higher than 1 MHz. The US transducer is composed of piezoelectric crystals capable of transforming electrical signals into US pulses (US waves) and vice versa (1). When the US pulses are sent into the tissues, a portion of the US energy is reflected back to the transducer at tissue interfaces. The portion of US energy that is transmitted further into the tissue allows the visualisation of deeper structures. The reflected US energy, which is dependent on the acoustic impedance (density x velocity), is received by the transducer and then transformed back into electrical signals. These signals are then displayed as dots on the screen (monitor) of the scanner. The brightness of these dots is proportional to the amplitude of the reflected echoes, while their location is proportional to the return time of the echoes.

US modalities

There are several US modalities that are used in clinical practise, each with specific properties that enable it to perform certain tasks. A-Mode (Amplitude mode) is one of the basic applications of US in medicine. It is a one-dimensional (1D) modality in which the signals are displayed as spikes on a horizontal sweep. The amplitude of these spikes is directly proportional to the strength of the echoes, while the location indicates the depth. The M-Mode (Motion mode), a further development of the A-mode, can be considered as A-mode displayed over time. B-Mode scanning (Brightness mode) is a two-dimensional (2D) method

with which a cross-sectional area of the tissue is visualised in real time. It is like several A-mode images put side by side in real time to cover a whole tissue region. This modality is now used in almost all clinical specialities. 3D B-Mode is used for volume estimation and complex organ reconstruction. It has been used to estimate the volumes of the stomach, liver and kidney (2-5). Volume estimation of tumours has also been made (6). Doppler US is a modality used for measurement of the velocity of blood and moving tissues. Doppler US utilizes the Doppler effect to measure velocities. The Doppler shift is the change in wave frequency (Δf). If the velocity of sound in the medium is known, then the Doppler shift of blood can be calculated from the formula:

$$\Delta f = \frac{2 f \bullet v \bullet \cos\theta}{c} \qquad (1)$$

Where Δf = Doppler shift, f = transmitted frequency, v = blood velocity, c = velocity of sound in blood, θ = angle between US bean and blood direction. From this equation, the velocity of blood can be calculated using the formula:

$$v = \frac{\Delta f \bullet c}{2 f \bullet \cos\theta} \qquad (2)$$

In medical US, the US waves are sent towards the moving blood and the received signals are used to calculate velocity.

Doppler Based Tissue Velocity Imaging

Tissue Doppler Imaging (TDI) is a Doppler method that is used to quantify myocardial velocities rather than blood velocities by allowing low- velocity information from the myocardium to be analysed (7;8). TDI includes both measurement of tissue velocity (Tissue Velocity Imaging - TVI) and measurement of strain rate (Strain Rate Imaging - SRI).

5.2 Strain Rate Imaging

Strain in physics

In mechanics, strain is defined as the deformation of a body or a structure as a result of an applied force (stress) (9). Deformation is measured in terms of either stretch from the formula: $\lambda = L/Lo$ (3)

or strain from the formula: $\varepsilon = (L - Lo)/Lo$ (4)

Where λ is stretch, ε is strain, Lo = baseline length and L is the instantaneous length at the time of measurement. From equation 1 and 2 we find that strain and stretch have the following relationship: $\varepsilon = (L\text{-}Lo)/Lo = L/Lo\text{-}1 = \lambda\text{-}1$ (5)

This strain is called the Lagrangian strain which can not be calculated unless both the initial length and the instantaneous length are known. Using Lagrangian strain, lengthening is positive strain and shorting is negative strain (Fig. 1):

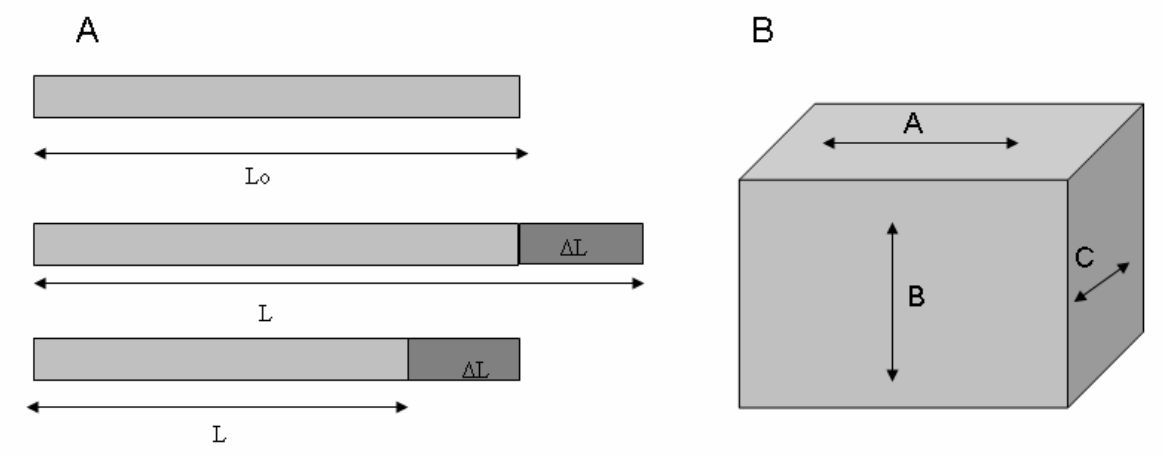

Fig. 1A. An illustration showing strain in a simplified one-dimensional object. Deformation is only possible as elongation (positive strain) or shortening (negative strain). Lo is the initial length, L is the instantaneous length and ΔL is L-Lo. Fig. 1B. An illustration showing strain in a 3D structure. There are 3 principal strain directions: D, L and W.

Another form for strain is the natural strain, which is defined as $\varepsilon_{nat} = (L - Lo)/L$

In most studies using SRI in cardiology the Langrangian strain is given (10).

The principal strain directions

In all 3D objects such as all biological tissues, there are three principal strain directions (Fig. 1B). In a tubular structure such as the antral wall, one of these directions is the circumferential direction. This is the direction along the curvature of the muscular wall. The second direction is the radial direction that runs across the wall thickness. The third direction is the longitudinal direction that runs parallel to the long axis of the stomach and perpendicular to the first two directions (Fig. 2).

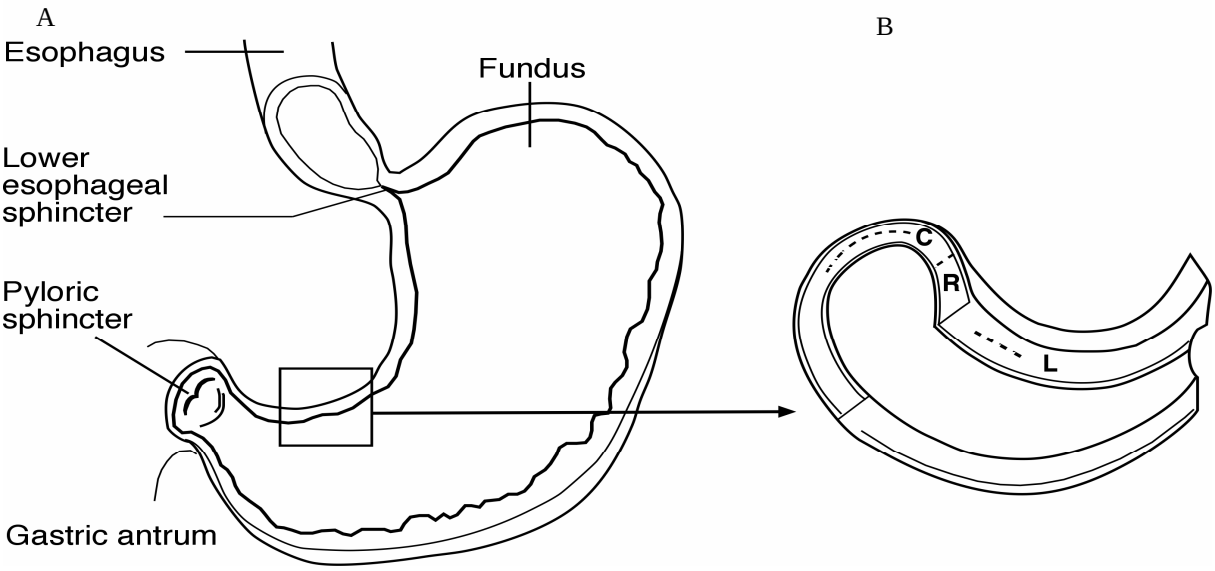

Fig 2. The orientation of the principal strain directions at one region of the human antrum in relation to the whole stomach. C, R and L in panel B are the circumferential, radial and longitudinal directions, respectively.

Strain in medicine

The deformation of a biological tissue can be a passive process whereby the tissue is subjected to a natural biological force, such as the deformation of the intra-abdominal organs due to pulsation from the aorta. This phenomenon is currently used for the diagnosis of pancreatic masses with elastography (11). Passive tissue deformation can also result from the application of an external force such as balloon distension of the gut (12). However, active deformation is a common physiological process. This is clearly seen in the myocardium and gastrointestinal tract. SRI in cardiology is used to assess the viability of the myocardium (13).

How does SRI work?

When an object deforms, elements within this object move. If the object is compressed, the elements will move towards each others and they will move away from each other when the object expands (Fig. 3).

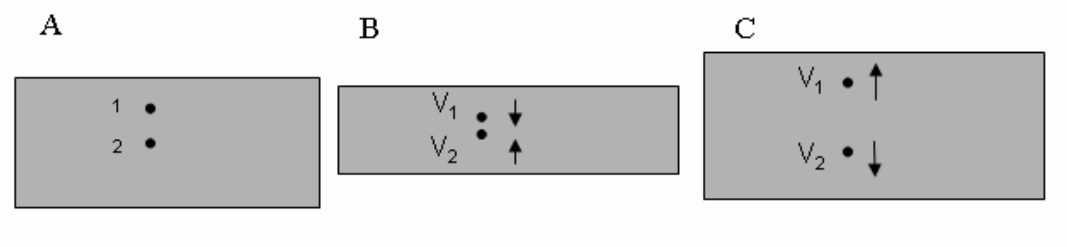

Fig. 3A. The points 1 and 2 are stationary. They move towards (panel B) and away from each other (panel C) with velocities V_1 and V_2.

The elements move towards or away from each others with certain velocities depending on the rate of deformation. The SRI, since it is a Doppler-based method, is able to measure the velocities of these elements and from their velocity gradients the strain rate is calculated and displayed as curves. The time integral of strain rate yields strain, which also can be displayed as curves (Fig. 4).

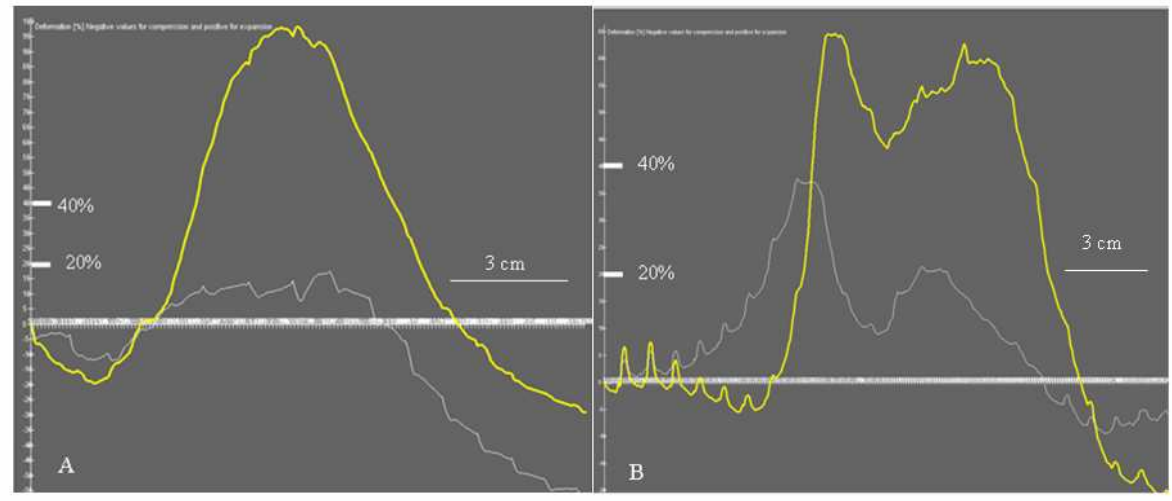

Fig. 4. Curves of anterior radial strain during a simple antral contraction (A) and during a complex antral contraction (B). Time is displayed on the x-axis and strain on the y-axis.

Important concepts

The strain length:

In theory, the SRI can measure strain from any two points within a deforming object. However, the distance between the points which velocities are used in the calculation of strain rate and strain should be chosen before starting the acquisition of the SRI cineloops. This distance is called “strain length”. In the scanner (SystemFive, GE Vingmed Ultrasound, Horten, Norway), the available choices for the strain length extend form 0.4 mm to 4.2 mm.

The selection of the appropriate strain length for the tissue thickness under study is important to obtain strain rate and strain values that represent the whole thickness. Choosing too small or too large strain lengths would result in less representative results. This is further explained in the Fig. 5. Ideally, the strain length should be ½ the wall thickness (14).

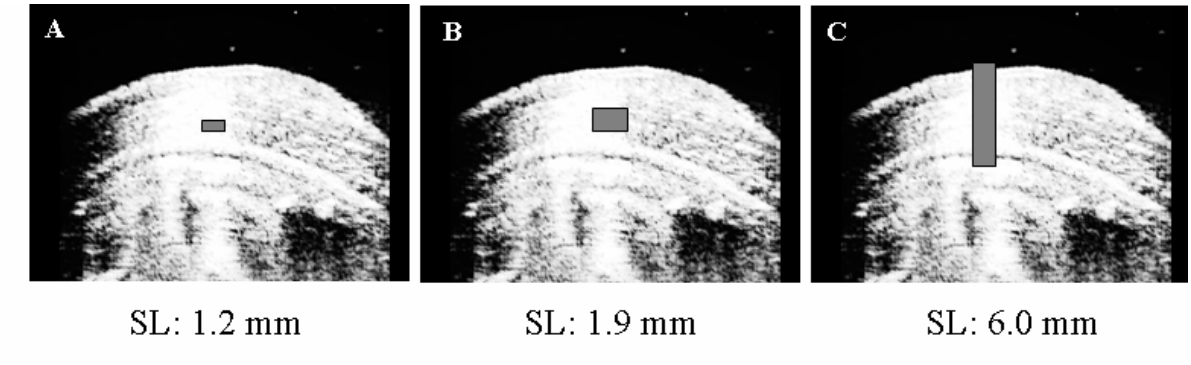

Fig. 5. Ultrasonograms of the anterior antral wall. The grey box represents the strain length. In panel A, the strain length is too small to represent the whole wall thickness. In panel B, the strain length is appropriate for measuring average strain across the wall for this wall thickness. In panel C, the strain length is too big and extends far beyond the wall limits.

The incompressibility theory

An incompressible object maintains its volume when it deforms. Change in one dimension is compensated for by change in the other directions. Assuming the antrum as being incompressible, positive radial strain is accompanied by negative circumferential and

longitudinal strains and vice versa (15).The incompressibility equation defines the following relation between stretch in the principal directions: $\lambda c \lambda r \lambda l = 1$ (6)

Where c, r and l are the circumferential, radial and longitudinal directions, respectively.

5.3 The motor function of the stomach

The main function of the stomach in the process of digestion is to store the food received from the oesophagus and to deliver this food to the duodenum. To accomplish this role, the stomach uses secretion of specific enzymes that facilitates the fragmentation of the ingested food into smaller particles (chyme). However, the most important factor involved in gastric function is its motility that enables the relaxation of its proximal part upon ingestion of food and the grinding, mixing and propelling of chyme through the narrow pylorus by the contractions of its distal part. Functionally, the stomach can be divided into two parts. The proximal part includes the corpus and the fundus. It is responsible for the storage of food entering the stomach. With relaxation being its main function, this part of the stomach has a thin muscular layer consisting of inner circular, middle oblique and outer longitudinal layers. The distal part (the antrum) has a thick muscular wall layer consisting of thin outer longitudinal and thick inner circular layers. The stomach is innervated by the vagus nerve and the enteric nervous system.

The motility pattern during fasting

After about 80% of the last meal has been emptied into the duodenum, the stomach enters the interdigestive state or fasting state in which the migrating motor complex (MMC) starts, and it is only terminated by the ingestion of food (16).

The MMC is a distinct cyclic motor activity that takes place in the stomach and small intestines during fasting (17). Each cycle consists of a phase with complete motor quiescence (phase I) that lasts for about 20- 40 min. Then phase II starts with irregular contractions, both in amplitude and frequency (less than 3/min). The contractions gradually increase in amplitude and frequency to 3 contraction/min (the maximum frequency of the pacemaker rhythm), giving rise to phase III, which lasts for 2-10 min, with great inter-individual variation (18;19). Then, everything falls into quiescence again in phase I. The contractions originate mainly in the proximal stomach. Some contractions fade away before they reach the antrum/pylorus, while others originate in the antrum (20). It is thought that the main function of the MMC is to clean the stomach and intestines of any food remains from the last meal, thus the term “the house keeper”. Trans-pyloric flow during fasting occurs in a similar fashion as observed in the fed-state (21). The highest rate of coordinated contractions occurs in phase III. This is accompanied by reduced resistance in the pylorus due to the action of motilin (22). Undigested food particles are then forced out of the stomach. Disruption of the MMC is associated with bacterial overgrowth (23). The gold standard for studying the motility pattern during fasting is manometry, which consists of a tube with several side holes for pressure measurements. The tube is placed in the stomach either through the nostril or the mouth.

Postprandial motility pattern

Upon swallowing, the motility pattern in the stomach during fasting ceases and the postprandial motor activity starts. This motor activity includes the relaxation of the proximal stomach, while more distally the stomach contracts to mix, grind, and propel the food into the duodenum. These two activities are discussed below:

Gastric accommodation

Accommodation is the process by which the proximal stomach relaxes (by the reduction of gastric tone and increase in compliance) to accommodate a meal without a corresponding increase in the intragastric pressure (24;25). The gastric accommodation reflex is usually divided into receptive and adaptive relaxation. Receptive relaxation was first described in 1911 by Cannon and Leib (26). It begins within seconds of ingestion, as the food approaches the oropharynx. This is a vagally mediated reflex. Adaptive accommodation is a relaxation that occurs after the food has reached the stomach (27). This is a gastro-gastric reflex which is induced by the mechanical distension of the stomach by food (28). A further relaxation occurs when nutrients reach the duodenum (29), a reflex mediated by the vagus nerve. Primarily, accommodation is the function of the proximal stomach, but some degree of postprandial relaxation takes place also in the antrum (30).

The barostat is considered the gold standard for the measurement of gastric accommodation. It consists of an intra-gastric polyethylene balloon compliant up to 1.0-1.2 L. The balloon is connected to a barostat device via a double lumen tube. The barostat keeps the balloon in contact with the wall through isobaric volume fluctuations. A 2D US-based technique has been developed to non-invasively assess gastric accommodation (31;32). Other methods used for measuring gastric accommodation include single-photon emission computed tomography (SPECT) (33), satiation drinking tests of liquid meals (34) and water load tests (35).

Gastric emptying

This can be defined as the process by which the intragastric content is delivered into the duodenum. Gastric emptying involves a well-organised coordination between the antrum, the pylorus and the duodenum (36). Generally, transpyloric transport occurs when the open pylorus leads to the formation of an antro-pyloro-duodenal common chamber (37). Within

this chamber, flow across the pyloric opening is bi-directional, nevertheless, the majority of flow events are forward (38-40). The proximal stomach plays an important role in controlling transpyloric flow of liquid meals, while “the antral pump” is important for emptying of solid meals (41;42). Scintigraphy is the gold standard for measuring gastric emptying for both liquids and solids (43). Other methods include breath test in which a ^{13}C-labbeled meal is ingested and then the concentration of ^{13}C is measured in the breath (44). A similar approach using acetaminophen (paracetamol) has been used (45). US is useful for assessing gastric emptying of liquid meals (46;47).

5.4 Functional dyspepsia

The definition of functional dyspepsia (FD) has evolved with the increasing knowledge and understanding of the mechanisms involved in its pathogenesis. The latest definition was provided by the Rome III classification of functional gastrointestinal disorders which defines FD as the presence of one or more dyspepsia symptoms that are considered to originate from the gastroduodenal region, in the absence of any organic, systemic, or metabolic disease that is likely to explain the symptoms. Rome III further identifies two subgroups of functional dyspepsia: “epigastric pain syndrome” (EPS) with the main symptom being epigastric pain which may or may not be related to intake of meals and “postprandial distress syndrome” (PDS) which is a bothersome postprandial fullness or early satiation (48). International studies estimated the incidence of FD at 1% per year (49;50). In a Norwegian community, the prevalence of non-ulcer dyspepsia was found to be 12 % (51). The pathophysiological factors underlying functional dyspepsia are poorly defined. Functional dyspepsia is largely attributed to disorders of the sensory and motility functions of the stomach. The most important pathophysiological factors are:

Impaired accommodation: Studies with US, barostat, scintigraphy and SPECT have shown that 40-60 % of FD patients have impaired gastric accommodation (52-56). It has been shown that impaired accommodation of the proximal stomach is associated with intragastric mal-distribution of meals, with pooling of the ingested meal in the antrum (57;58).

Delayed gastric emptying: Delayed gastric emptying for liquids was found in 35% and for solids in 23% of FD patients (59). In a subset of FD patients, rapid initial gastric emptying of solids, liquids and saline has been reported (60;61).

Hypersensitivity to distension: It has been observed that FD patients have hypersensitivity to balloon distension of the stomach that is not related to impaired accommodation (62;63). Cerebral evoked potentials suggested that FD patients may have abnormal central processing of visceral perception (64). Hypersensitivity to distension was found to be associated with epigastric pain, belching and weight loss (65).

Other factors that have been shown to be involved in the pathogenesis of FD include gastric dysrhythmia, abnormal antroduodenal motility, altered sensitivity to duodenal lipid or acid exposure, Helicobacter pylori infection and psychological factors

Treatment

Because of the diversity of both the clinical presentation and pathophysiological factors involved, no single treatment strategy has been developed for FD. Some patients benefit from acid suppression by histamine receptors (H2) blocker and proton pump inhibitors (PPI) and from prokinetic agents (66), while others may benefit from other treatments such as low doses antidepressants (67), visceral analgetics (68), herbal preparations (69), psychotherapy (70) and hypnotherapy (71).

6.0 Aims of the study

The overall aim of this work was to assess the feasibility of SRI in assessing antral contractility in FD patients and healthy controls. The specific aims of the individual studies were:

Study 1:

To evaluate ultrasound Doppler strain rate imaging (SRI) in vitro using a silicone strip phantom mimicking slowly moving tissue.

Study 2:

To evaluate the accuracy of the SRI method in measuring strains in the porcine antral wall in the radial and circumferential directions in vitro, in comparison with strain calculated from B-mode and sonomicrometry.

Study 3:

To characterise radial strain of the proper muscle layer of the antral wall during different phases of the MMC during lumen-occlusive versus non-lumen occlusive contractions, postprandially and after erythromycin. To evaluate the relationship between antral strain on one hand and gastric emptying and proximal gastric accommodation on the other hand.

Study 4:

To explore the differences in antral radial strain measured by SRI in FD patients and healthy controls, both fasting and postprandially. To find the relationship between strain and gastric accommodation, gastric emptying and visceral hypersensitivity.

7.0 Materials and Methods

7.1 Materials

Ethical aspects

The participants in studies III and IV gave written informed consent. The protocol was approved by the Regional Committee for Medical Research Ethics and the procedures were carried out according to the revised Helsinki declaration. In study II, the porcine stomachs were removed from recently scarified pigs from other ongoing studies with approved animal protocols.

The elastic rubber phantom (Paper I)

A 5 mm thick phantom was made of silicone rubber, a completely elastic material (72). The phantom was used to simulate the muscular layer of the antral wall. The rubber phantom's distribution of scatterers was similar to intact muscle. The velocity of US in the silicone rubber was close to 1000 m/s compared to the calibration of most scanners, which use 1540 m/s (velocity of saline at 37.5°C) (73;74). Consequently, B-mode measurements of length were corrected by a factor (1000/1540). On the other hand, strain rate and strain values would not be affected by change of US velocity because they are relative measures (10).

The porcine stomach (Paper II)

Ten stomachs from pigs (weight: 45-55 kg) were harvested and cleansed with water. The stomachs were cut at about 15 cm proximal to the pylorus on both the lesser and the greater curvatures. The distal ends were cut and tightened 1-2 cm distal to the pylorus, while the proximal parts were tightened around a plastic disc with 2 holes. One of the holes was connected to the infusion tube and the other hole was used to insert the pressure catheter.

The healthy volunteers (Paper III and paper IV)

Characteristics of the healthy participants are listed in table 1. The healthy participants did not have any known disease and no symptoms of gastrointestinal disease. FD was excluded by the Rome II criteria after completing a questionnaire.

FD patients (Paper IV)

Characteristics of the FD patients who participated in the clinical study are also listed in the table below. They fulfilled the criteria for FD according to Rome II and Rome III. They were allocated one of the two subgroups of FD according to the criteria of Rome III: 5 EPS patients and 11 PDS patients.

Table. The demographic characteristics of FD patients and controls.

	Study III	Study IV		
	HV	FD	Controls	*P*
Female/male	4/3	12/4	11/4	
Age (years): median (range)	27.1 (19-37)	33.0 (19-58)	32.00 (19-58)	>0.05
Height (m): mean (SD)	1.75 ± 0.1	1.71 ± 0.1	1.73 ± 0.1	>0.05
Weight (kg): mean (SD)	65.1 ± 7.3	65.9 ± 13.9	66.3 ± 3.1	>0.05
BMI (kg m^{-2}): mean (SD)	21.2 ± 1.3	22.4 ± 3.5	22.0 ± 2.3	>0.05

HV: healthy volunteers. FD: patients with functional dyspepsia. *P*-values show level of significance between FD and controls

7.2 Methods

Acquisition of SRI cineloops (All papers)

In all the studies, a digital US scanner (SystemFive, GE Vingmed Ultrasound, Horten, Norway) with custom acquisition software was used. To obtain strain rate cineloops, a linear

probe was used, utilizing a tissue frequency of 6.7 or 10.0 MHz and a Doppler frequency of 5.7 or 6.7 MHz. During acquisition, a pulse repetition frequency of 0.25 kHz was used to decrease the Nyquist strain rate (maximum measurable strain rate) to 6.1 s^{-1}, to reduce the scaling error introduced by these relatively low strain rates compared with those of contracting myocardium (75). The strain length was set at 1.9 mm in studies III and IV, since results from studies I and II showed it was the most suitable for a wall thickness in the magnitude of that of the antral wall. The strain rate and strain along the US beams were displayed by colour coding of a window superimposed on the B-mode images (Fig 6).

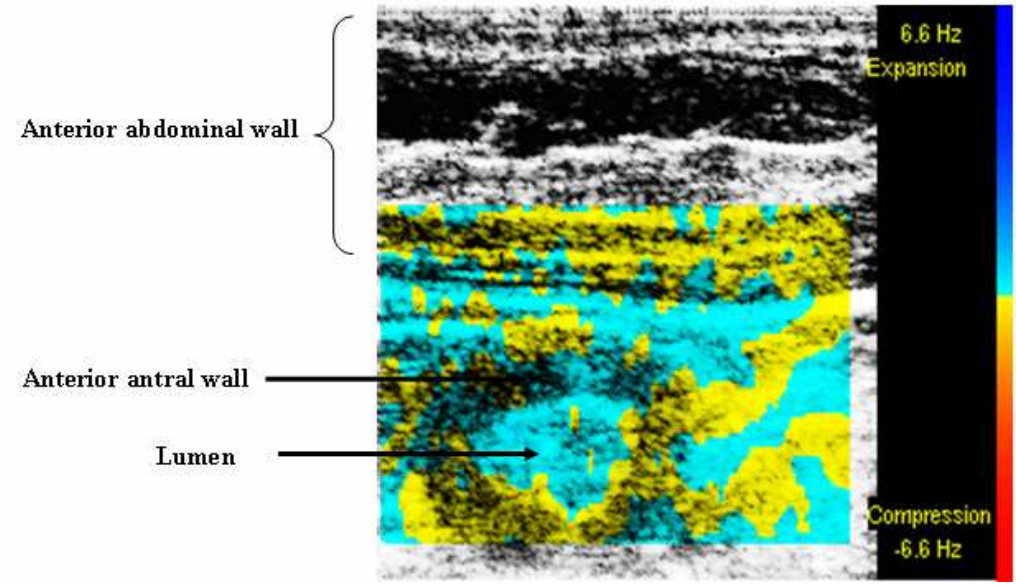

Fig. 6. Doppler colour coding of SRI in the human antrum. A turquoise to blue colour indicates positive strain and yellow to red colour indicates negative strain (right margin).

After transferring the cineloops from the scanner to a PC, measurement of strain was done using dedicated software (TVI.exe version 6.1, GE Vingmed Ultrasound, Horten. Norway). This software enables the operator to compute strain either from single point samples, by averaging strain along the beam or across several US beams, or in a curved line that averages the strain over the whole tissue strip. In study I, we found that averaging strain over the whole strip was not significantly superior to averaging across several US beams and it is time-consuming. Thus, we used the method of averaging across several US beams in

studies II-IV. The result of the analysis is displayed as a curve from which different parameters can be obtained, such as peak strain, time to peak, time to baseline and total contraction time.

Measurement of strain from B-mode (Papers I & II)

The accuracy of SRI in assessing radial strain was compared with strain calculated from B-mode of the silicone phantom in study I and from B-mode of the porcine anterior antral wall in study II. In study I, the thickness of the whole silicone phantom was measured. For the antral wall in study II, however, only the thickness of the muscularis propria from the serosa to the second interface echo between the muscularis propria and submucosa was measured from B-mode images. Strain was calculated using formula (2).

The US scanning for gastric accommodation (Papers III & IV)

The same scanner (SystemFive, Vingmed Ultrasound, Horten, Norway) used for obtaining the SRI cineloops was used to obtain images of the proximal stomach by the method described by Gilja et al (76). A curvilinear US transducer (3.5 MHZ) was held vertically in the epigastrium to identify the proximal stomach between the liver and the left kidney. Images were saved according to the protocol (first in the fasting state about 5 min before ingestion of the test meal and at 1, 10, 20 and 40 min postprandially) and were then transferred to a PC for the offline area measurements using commercial software (EchoPac3D, GE Vingmed Ultrasound, Horten, Norway). The measurement method used is shown in Fig. 7, where a 7 cm long line was drawn form the fundus to the middle of the corpus of the stomach. Then the outer surface of the proximal stomach was traced within this line. Fig. 7 also shows the area of the proximal stomach during fasting in a healthy volunteer and in a FD patient; and the area of proximal stomach at 1 min postprandially in a healthy volunteer.

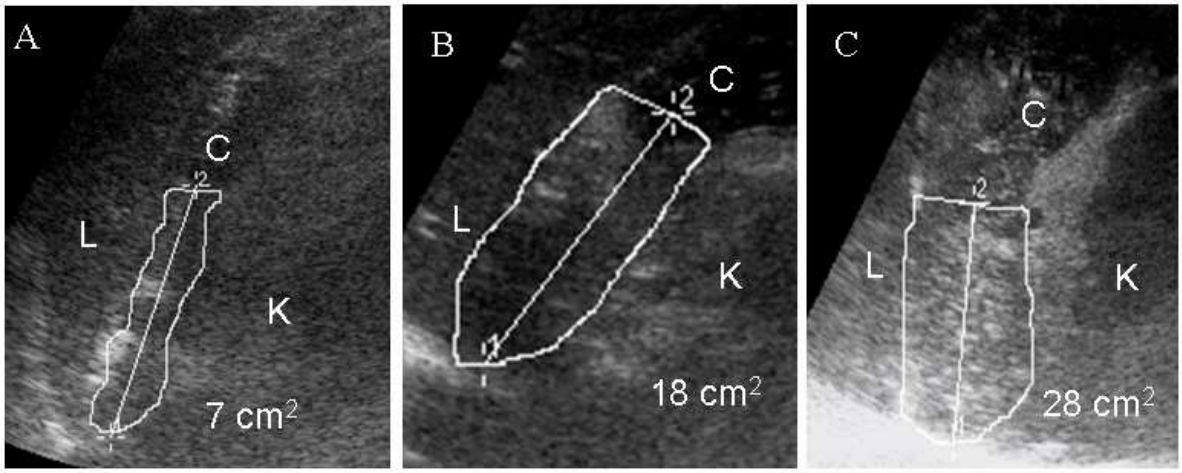

Fig. 7. Ultrasonograms of area measurements of the proximal stomach. The 7 cm line is drawn from the fundus towards the corpus. The gastric cavity is outlined by the curved line and the measured area within the curved line is used as the area of the proximal stomach. The proximal area during fasting is shown in a healthy individual (panel A) and in a patient with FD (panel B). Panel C shows the proximal area at 1 min postprandially in a healthy individual. L= liver. C= corpus. K= left kidney.

The US scanning for gastric emptying (Paper III & IV)

Gastric emptying was measured by using a previously validated US-based method (77;78). The images of the antrum were repeatedly obtained just after obtaining the images of the proximal stomach according to the protocol. In a sagital plane at about 2 cm proximal for the pylorus and including the liver anteriorly, the antral CSA was identified by the linear US transducer used for acquiring SRI cineloops. The images were then saved and subsequently transferred to a PC for the offline measurement of antral CSA using EchoPac3D ®. The antral CSA was measured by tracing the inner limit of the muscularis propria.

Sonomicrometry (Paper II)

Sonomicrometry measures the distance between two piezoelectric crystals inserted into soft tissues. The method has been used as a gold standard in cardiovascular research for the measurement of myocardial function (79). It has also been used to study the gut motility (80).The physical basis of sonomicrometry is the measurement of the time required for an US pulse to travel between a transmitter and a receiver crystal, both implanted into the tissue

which motion is to be studied (81). In paper II, two pairs of piezoelectric crystals were implanted 5-10 cm proximal for the pylorus, on the lesser curvature, one pair along the longitudinal axis and the other in the circumferential direction, perpendicular to the first pair (Fig. 8A). The crystals were connected to a sonomicrometer (type 500, Triton Technologies, San Diego, CA, USA) that displayed the distance between the crystals as curves (Fig. 8A). Strain then was calculated from formula (2).

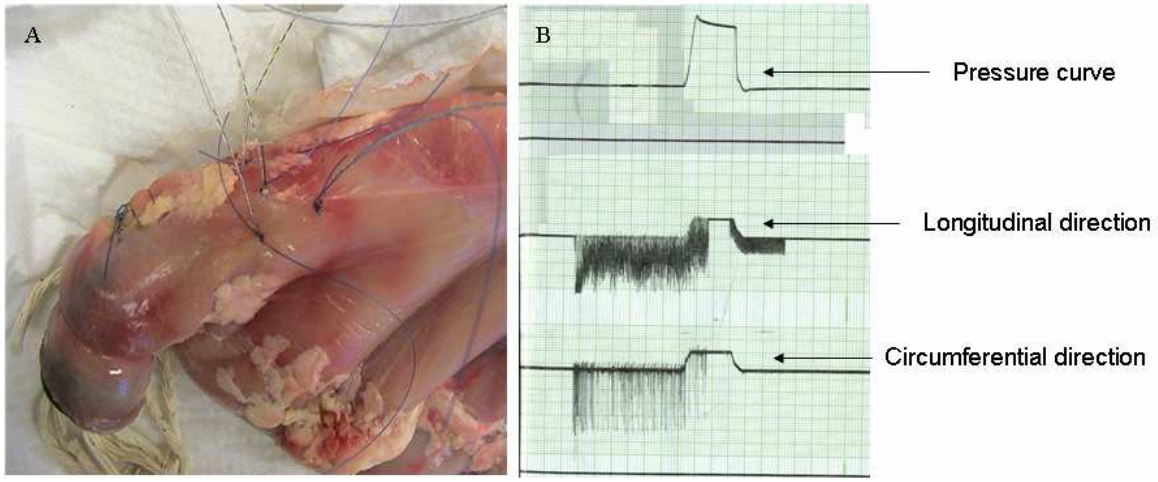

Fig. 8A. Sonomicrometry crystals inserted into the antral wall. The curves in Fig. 8B show the instantaneous change in intragastric pressure and in distances between the longitudinal and circumferential pairs of sonomicrometry crystals. The interaction between the two US systems generated some noise on the sonomicrometry curves.

Pressure measurement (Paper I1)

Through the smaller hole in the plastic disc in which the proximal part of the porcine stomach was tightened, a pressure catheter (Millar Mikro-Tip ®; model: PC-350; size: 5F. Millar Instruments, Houston, Texas, USA) was inserted into the lumen of the stomachs. The pressure transducer was attached to an amplifier (Hewlett Packard 88050, Waltham, MA, USA) connected to a printer (Hewlett Packard 7754 B) that displayed pressure in real time on a curve (Fig. 8B).

The test meal (Papers III and IV)

An amount of 500 ml of commercial meat soup (Toro clear meat soup, Rieber & Søn A/S, Bergen, Norway) containing 1.8 g protein, 0.9 g fat, and 1.1 g carbohydrate (20 kcal) (all soluble in water) was ingested during a period of 4 min. The soup was boiled and then cooled to 37º C to reduce the amount of air bubbles after ingestion. The pH of the soup varied between 5.4 and 5.7, and the osmolarity was 350 mOsm/kg H2O (82;83).

Symptom score and perception of hunger (Papers III and IV)

Each subjective abdominal symptom (pain, nausea, fullness, satiety and total discomfort) was assessed by a visual analog scale (VAS) consisting of a 100 mm long line with zero indicating "no perception" and 100 mm indicating "maximum perception" (84;85). The scoring was done about 5 min before ingesting the test meal and at 1, 10, 20 and 40 min postprandially. The symptom that was associated with maximal awareness of hunger was also registered.

8.0 Summary of results

Paper 1 (The elastic rubber phantom in vitro study)

SRI strains agreed well with calculated values for strain when SRI strain was measured as the average over the whole strip cross-section and the strain sample size (called strain length in papers II-IV) was 1.9 mm (mean difference = 2.78 %, limits of agreement ± 9.97 % for tissue velocity 1.0 mm/s, n=8). Averaging over the whole strip was not significantly superior to averaging across several US beams and it was time consuming.

Paper 2 (the porcine stomach- in vitro study)

The agreement between radial strain values measured by SRI and B-mode across several ultrasound beams, using US frequency 6.7 MHz and strain length = 1.9 mm was good (mean difference= 0.5%, limits of agreement ± 13.4%) and it was better than with strain length 1.2 mm.

Paper 3 (Study on healthy volunteers)

In the fasting state, anterior radial strain measured by SRI increased gradually from early to late phase II (P <0.001) and reached a peak during phase III activity (P <0.001). Postprandial strain values did not differ from that in the different phases of MMC. Anterior radial strain during contractions after the administration of erythromycin during phase I did not differ from that during spontaneous fasting contractions, though some erythromycin contractions had considerably high strain values. Postprandial anterior radial strain with erythromycin was lower compared with late phase II (P <0.05) and phase III (P <0.01) activity, but did not differ from postprandial strain after placebo. Erythromycin induced more frequent lumen-occlusive contractions, both fasting and postprandially. Erythromycin reduced the total postprandial area of the proximal stomach (P < 0.001).

Paper 4 (Clinical study – FD patients and controls)

EPS patients had higher anterior radial strain than controls ($p=0.036$) and than PDS patients ($p<0.001$) during fasting and postprandially states. FD-EPS exhibited different development of the strain in the MMC phases. In the fasting state, FD had significantly larger proximal area compared with controls (14.0 ± 1.4 cm^2 vs. 7.9 ± 0.2 cm^2, $p=0.001$). Compared with controls, PDS group had significantly larger (14.9 ± 1.6 cm^2, $p<0.001$) fasting proximal area, but EPS did not differ (11.9 ± 2.6 cm^2, $p>0.5$). The accommodation response ratio was significantly higher in controls than in FD at 1 and 10 min postprandially. Gastric emptying fraction was significantly larger at 20 and 40 min in controls than in FD. Analysis of variance (ANOVA) showed that EPS values for gastric emptying at 20 and 40 min were midway between those of controls and PDS, but not statistically significant from either. Both fasting symptoms score and area under the curve in the postprandial state were significantly higher in FD than in controls for all variables.

9.0 General discussion

9.1 FD and the need for non-invasive techniques

Globally, the prevalence of uninvestigated dyspepsia is estimated at 20%-30% (86-89). The majority of these cases have presumably a functional disorder (90;91). Most of the methods available for the study of FD are either invasive or of low temporal or/and spatial resolution and hence they are not suitable for gut motility. The invasive methods do not only cause some degree of discomfort to the patients, they may even disturb the normal function of the gut (92). These methods include the barostat, manometry and balloon devises to elicit certain reflexes or stimulate certain receptors. Non-invasive methods such as Magnetic Resonance Imaging (MRI) and scintigraphy are preferred by the patients. However, MRI is expensive, anxiety provocative, not widely available and it is not suitable for long investigations. Scintigraphy carries the risk of exposure to radiation. US can be used under long investigations. In this doctoral dissertation, we tested the application of a non-invasive, US-based Doppler modality, namely SRI, on the human antrum to investigate FD patients.

9.2 SRI and normal antral motility

The stomach motility during fasting is all about the migrating motor complexes. In paper III, we showed that radial strain in the anterior antral wall during contractions gradually increased from the beginning of phase II to reach a maximum in phase III, before quiescence appeared in phase I. This finding confirms previous results by manometric studies (93). The main advantage of manometry is that it is able to detect the speed and direction of contraction propagation over relative long distances. However, manometry detects only lumen-occlusive contractions (94). Therefore, contractions taking place in the proximal stomach, most of which are non-lumen occlusive, would not be detected by manometry. Manometry also has little value in assessing postprandial antral contractions, since the lumen-occlusive

contractions in the postprandial state become less frequent (95).The method is also invasive, influencing normal physiology. In this context, US is a non-invasive method that is more sensitive than manometry in detecting antral contractions (96;97).

Other modalities used for the assessment of gastric contractions include the barostat, which detects volume fluctuations caused by these contractions, but no further information on localisation, number or propagation direction of the contractions can be provided (98). Other visualisation modalities used for the assessment of antral motor activity include scintigraphy, which has been shown to be more sensitive than manometry in the detection of antral contractions, especially proximally, where these contractions are usually non-lumen occlusive (99). However, scintigraphy is associated with exposure to radiation and should not be performed repeatedly. Antral motility during fasting can not be studied by scintigraphy because a radioactive-labelled test meal (Technetium-99m) must be ingested by the subjects. No published studies that compare MRI and scintigraphy with US in studying gastric contraction are available. The advantage of MRI is its ability to assess the motor activity of both the proximal stomach and the antrum simultaneously (100). However, the temporal and spatial resolution of MRI has been considered insufficient for the detection of transient or small wall movements (101). MRI can be used for assessing postprandial contractions, but an important limitation of MRI is the need for intraluminal contrast (meal) that often resides in the proximal stomach, since most MRI scanners operate on subjects in the supine position (102). New MRI scanners that give better temporal resolution have been developed, but they are not widely available. US carries no radiation burden and is widely available worldwide. Furthermore, US has the advantages of being non-invasive and it exhibits high spatial and temporal resolution. Therefore, US is a valuable tool for assessing gastric motility both during fasting and postprandially.

9.3 The accuracy of SRI in the antral wall

The SRI method was initially developed to assess myocardial function. The heart has much more rapid contractions than the gastric antrum. In paper I, we wanted to find out whether the SRI could be modified to accurately measure strain associated with slower contractions and what parameters should be adjusted. First, the filters were adjusted to include the Doppler shifts from the antral wall. This was done adjusting both high- and low-pass filters. Then the adjusted method was used to find out whether strain and strain rates from slowly moving tissues could be measured. For this purpose, we used a rubber phantom comparable to the smooth muscle in terms of US properties, the degree of inhomogeniety and thickness. The main parameters we considered in this study were the velocity of deformation of the silicon rubber phantom, strain length and method of averaging. To assess the suitability of the rubber phantom for this particular purpose, the stress-strain relation was calculated and it gave an elastic modulus of 825 kPa, the same order of magnitude of the gastrointestinal tract (103). Thus, the subsequent results were considered appropriate for the evaluation of strain in the antrum.

The main result of the study was the finding that strain measured by SRI showed good agreement with strain calculated from B-Mode. However, strain measured by SRI showed some underestimation. This underestimation was especially present when a very low tissue velocity was used (0.1 mm/s). In contrast, the velocity of 0.5 mm/s and 1.0 mm/s gave acceptable results. Tissue velocities in the human antrum are unlikely to be as low as 0.1 mm/s. We also found that strain length of 0.8 mm was too small for the rubber phantom, while strain length of 1.9 mm gave better results. Choosing the appropriate strain length for the tissue under assessment is important. Since the thickness of the rubber phantom was similar to that of the human antrum, strain length of 1.9 mm was applied in the studies on

volunteers and FD patients. A previous study on the myocardium showed that the strain length should be set at approximately half the thickness of the studies tissue (104).

Although the results from the first study were promising, the rubber phantom strip did not represent the human antrum in terms of configuration and wall layers. Therefore, we applied SRI on the intact porcine antrum in study II to evaluate the accuracy of the SRI method in measuring strains in a biological tissue that is representative for the human antrum. The study showed a good correlation between strain measured by SRI and strain calculated from B-mode. The study also confirmed that strain length 1.9 mm was more suitable that strain length 1.2 mm for the porcine antral wall.

The human studies (papers III & IV) showed that the strain values had non normal distribution and large variance. Non parametric statistical methods were used to analyse these data. The strain values in controlled deformation of a rubber phantom (paper I) and biological tissues (paper II), showed less variance. We believe that the large variance of strain values in the human studies resulted from normal physiological variation of the contractions. It is well-known that there is a great intra-individual variation in the amplitudes of gut contractions.

9.4 SRI and strain in FD

We used SRI in a cohort of FD patients who were allocated to one of the two subgroups of FD according to Rome III, namely “epigastric pain syndrome” and “postprandial distress syndrome”. EPS patients were found to have higher strain values than both healthy controls and PDS patients, both during fasting and postprandially. PDS patients had lower strain than controls. Both strong and weak contractions have been observed in FD patients. Since it has only been recently introduced, previous reports did not take into consideration the Rome III subgroups of FD. This gives difficulties in comparing our results with previous data. Stomach dysmotility as demonstrated by delayed gastric emptying is a recognized pathophysiological

factor in FD (105). The prokinetic agent erythromycin improved gastric motility and delayed gastric emptying, although it does not improve symptoms (106). Erythromycin is a motilin agonist that enhances contractions of the stomach (107).

In a subset of FD patients, unsuppressed proximal stomach contractions have been observed by the barostat (108). Whether these patients had EPS or not, is not known. Our study supports that finding in FD, with the additional value of confining the finding to EPS patients. Higher strain means greater degree of deformation which indicates stronger contractions. Recent studies provide evidence that the mechanoreceptors responsible for symptom generation are stimulated by the mechanical deformation of the tissues (109-113). We believe that higher strain levels in EPS patients stimulate mechanoreceptors, resulting in pain sensation. It has been observed that some FD patients have hypersensitivity to balloon distension of the stomach that is not related to impaired accommodation (114;115). This means that these patients have lower threshold for the distension stimuli. Another study with cerebral evoked potentials suggested that FD may have abnormal central processing of visceral perception (116). Our study indicates that there is excessive stimulation of mechanoreceptors by higher levels of strain, regardless of the level of sensory threshold.

9.5 Gastric accommodation and emptying

A novel ultrasonographical finding in our study is a larger proximal area of the fasting stomach in FD patients. Previous US studies provided evidence that FD patients have a wider antrum (117). In paper IV, we could only find a trend for a wider antrum in FD, but no statistically significant difference from controls. However, we measured the CSA of the antrum at 1-2 cm proximal to the pylorus, while other studies measured the antral area up to 5 cm proximal to the pylorus (118). This may indicate that the total fasting stomach volume in FD is larger than in healthy volunteers. Studies using SPECT confirmed this finding (119).

The barostat, considered the gold standard for measuring gastric accommodation, is not able to detect fasting intragastric volumes. In fact, the barostat may displace gastric content into the distal stomach (120).

Although proximal gastric volumes and areas previously have been measured by US in the postprandial period, this is, to our knowledge, the first study which measured the proximal gastric area during fasting. This approach enabled the calculation of the accommodation reflex in a manner similar to what is used in the barostat studies, namely by calculating the difference between the postprandial and fasting volumes. However, we used the approach used in studies with SPECT where the accommodation ratio is calculated by dividing the postprandial proximal area by the fasting proximal area. This approach was chosen because SPECT is also a visualization modality.

9.6 Erythromycin effect

In study III, we found that anterior radial strain during erythromycin-induced contractions did not differ from that during spontaneous fasting contractions. However, some erythromycin contractions had considerably high strain values. In the postprandial period, anterior radial strain during erythromycin-induced contractions was lower compared with late phase II and phase III, but did not differ from postprandial strain with placebo. Erythromycin induced more frequent lumen-occlusive contractions, both during fasting and postprandially. Erythromycin reduced the total postprandial area of the proximal stomach.

Previous manometric observations showed that erythromycin induces phase III-like contractions (121). We found that erythromycin-induced contractions were similar to contractions of phase II of the MMC. One possible explanation for that manometric finding is that erythromycin induced more frequent lumen occlusive contractions that are likely to be detected manometrically. Although postprandial contractions during erythromycin infusion

were not found to be different from during placebo infusion, the significantly increased frequency of lumen-occlusive contractions during erythromycin infusion could play an important role in erythromycin influence on the postprandial motility resulting in more rapid gastric emptying.

9.7 Limitations of SRI

The SRI method suffers the same limitations of other US modalities, namely that air and fat in the examined tissues or organs hamper visualisation. The main specific limitation of SRI is its angle-dependency. A detailed theoretical description of the angle-dependency of SRI has been provided by Heimdal (10). Angle-dependency of SRI has also been shown in a computer simulation and in vivo on cardiac muscle (122). As is reflected in equation (2), for the calculation of velocity by Doppler, the cosine of the angle between the US beam and the moving tissue is used. Strain is even more angle-dependent since the squared cosine ($\cos^2 \theta$) is used for its calculation (10). This implies that as the angle between the US beams and the muscle layer increases, the calculated velocity decreases. In a tubal structure like the antrum, this angle-dependency constitutes a great challenge. In theory, only the anterior and the posterior antral wall should be studied, since the US beams fall perpendicular to the wall and thus parallel to the radial strain direction. In practice, however, strain in the anterior wall is more easily accessible, since the visibility of the posterior wall is sometimes jeopardised by the presence of intraluminal air and food debris. Angle-dependency also makes estimation of circumferential strain less accurate using the SRI method. That is because the configuration of the antral wall in the circumferential direction changes under contraction and relaxation. Also in cardiology, few studies provide data of strain in the circumferential direction of the left ventricle, probably due to difficulties in obtaining good estimates (123).

10.0 Main conclusions

- For the SRI method to give accurate estimates of strain, the strain length should be set at 1.9 mm. Averaging over several ultrasound beams increase the accuracy further.

- SRI gave accurate measurement of radial strain of the ex-vivo porcine antral wall, but seemed to be less accurate for measurement of circumferential strain in *vitro.*

- SRI enabled detailed radial strain measurement of individual antral contractions, both during fasting and postprandially. Erythromycin initiated fasting antral contractions and increased the number of lumen occlusive contractions.

- Anterior radial strain measured by SRI non-invasively detects clinically relevant gastric motor abnormalities that discriminate between the two subgroups of FD according to the Rome III classification and healthy controls. This result supports the Rome III classification of FD into EPS and PDS groups.

11.0 Future prospective

Although this study showed that SRI is sensitive enough to detect physiological activities in the human antrum and to discriminate between healthy individuals and patients with FD, the limitation resulting from angle-dependency constitutes a major challenge in studying gastric motility. This results in the possibility of measuring strain mainly in the anterior wall.

In cardiology, however, a newer tissue imaging technique to measure strain has evolved, called speckle tracking echocardiography (STE - also called 2D strain), that enable simultaneous measurement of strain in the radial and circumferential directions at any given area. Since this technique is not angle-dependent, strain in the whole circumference can be measured simultaneously. In Fig. 9, an antral contraction is analysed by 2D strain. The circumference is divided into 6 segments. Circumferential strains (average of each segment) are displayed. However, the technique requires modifications of the scanner before it can be applied to antral contractions as a clinical tool.

The feasibility and the potential of 2D strain methods in the human antrum in healthy individuals and gastric motility disorders ought to be explored. This probably will enhance our understanding of physiological and pathophysiological processes.

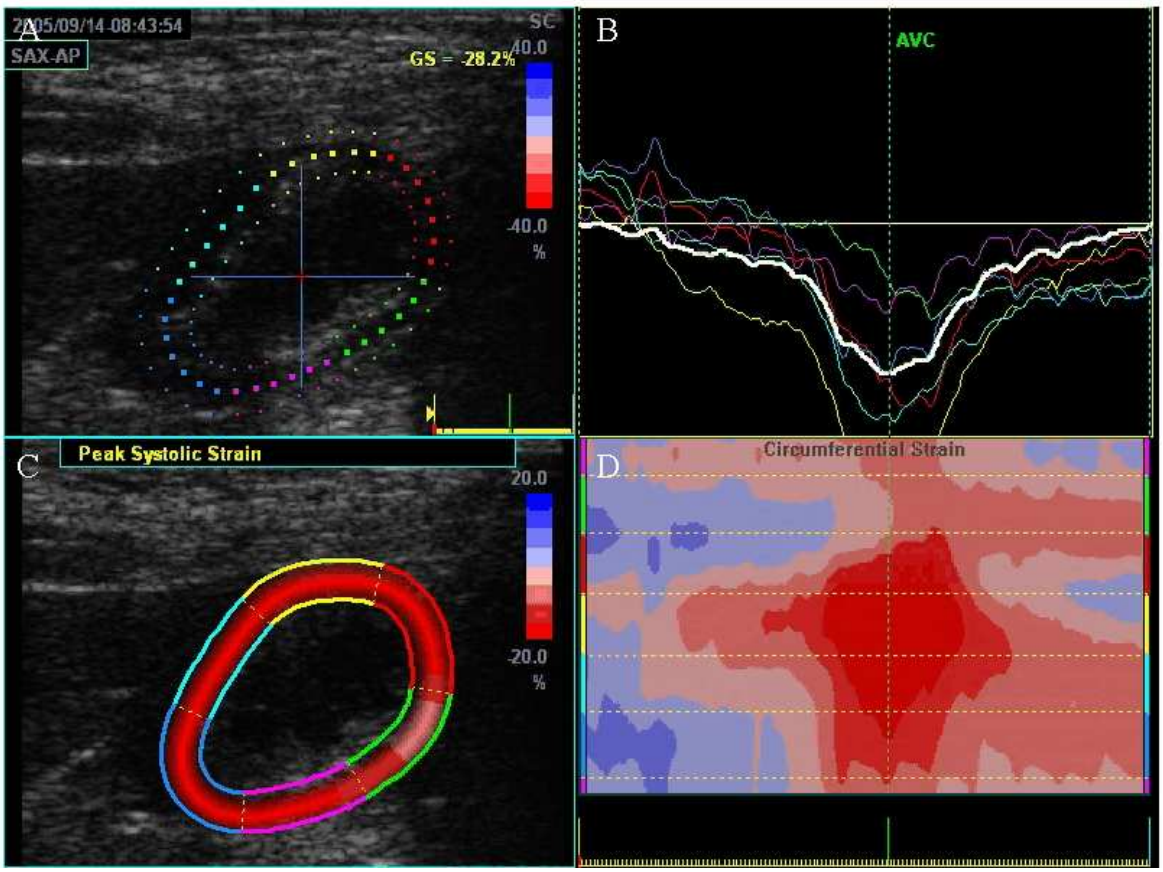

Fig. 9. 2D strain was used to analyse an antral contraction in a healthy volunteer. Circumferential strain is shown. Panel A shows the different segments of the antral wall. Panel B shows the strain curves for each segment. The colours of the curves correspond to the colours of the segments in panel A. In panel C, the colours show the instantaneous state of strain (negative or positive). The colour coding on the right shows that blue indicates positive strain (increase in circumference) and red indicates negative strain (decrease in circumference). Panel D is a coloured anatomical M-mode with the complete circumference as y axis and time along the x axis.

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